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Soft X-ray observation of water distribution in the stem of *Cryptomeria japonica* D. Don I: General description of water distribution*

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Abstract Water distribution in green stems of *Cryptomeria japonica* D. Don was observed by soft X-ray photography. In the sapwood, much water was present and evenly distributed. In the intermediate wood (the white zone), little water was present. The intermediate wood appeared in all cross sections of the stem and separated the heartwood from the sapwood in the intertracheid water connection. Maldistribution of water was generally observed in the heartwood, and three types of water presence were distinguishable: a "wet area" with accumulated water, a "dry area" with little water, and a "moderate moisture area" with intermediate accumulation. The distribution pattern and amount of water in the heartwood varied dramatically among and even within trees. Separation of the heartwood from the sapwood in the intertracheid water connection suggested that the presence of water in the heartwood was caused by rewetting of the tracheid lumina that occurred after heartwood formation. The maldistribution of water in the heartwood suggested that a difference in the process of rewetting causes both uneven distribution and the various types of water presence.

Key words *Cryptomeria japonica* · Wetwood · Soft X-ray photography · Water distribution · Heartwood

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Introduction

It has been reported that the moisture content of a living tree of coniferous species is generally high in sapwood and low in heartwood.[1,2] However, species of some genera (e.g., *Abies*, *Pinus*, *Larix*, and *Tsuga*) have portions of heartwood with higher moisture content than surrounding regions or sometimes even higher than the sapwood.[3–6] Portions of the heartwood with such high moisture content are termed wetwood.[4,6]

Cryptomeria japonica D. Don (sugi or japanese cedar) has been detected as a wetwood species, and the moisture content of the heartwood is known to vary among trees.[2,7] In addition, between-tree variation of moisture content related to a variation of heartwood color has been reported. Fujioka and Takahashi[7] investigated the variation of moisture content between black-heart and red-heart trees and reported that black-heart trees showed an obviously higher moisture content. Yazawa[2] also reported that the moisture content (percentage of dry weight) of the heartwood of the *C. japonica* tree trunk was 113.1% for black-heart trees and 72.4% for red-heart trees.

In some investigations the moisture content of *C. japonica* heartwood was shown to be uneven and varied within a tree. Miwa[8] and others[9–11] reported several types of radial distribution of moisture content in the heartwood. Some have pointed out considerable variation of moisture content in the heartwood among clones and cultivars but comparatively less variation within clones and cultivars of *C. japonica*.[9,11]

Such moisture variation between trees and within each tree makes log drying of *C. japonica* expensive.[12] Furthermore, black-heart, which lowers the market value of unfinished logs,[13] is strongly related to the presence of wetwood in *C. japonica*.[10] Wetwood may also be a cause of frost crack.[14,15]

The low value of moisture content in the heartwood of conifers is a result of dehydration of the tracheid lumen during heartwood formation. Many coniferous species showed a moisture content in the heartwood of 30%–

50%,[2,16] a slightly higher level than the fiber saturation point. This measurement indicates that there is little free water in the xylem lumen of the heartwood of those species. Therefore, the higher level of moisture content in the heartwood of the other coniferous species, which contains wetwood in the stem, suggests that free water is present in the xylem lumina therein.

Soft X-ray photography has been applied to the study of wetwood.[9,17–19] This method is more easily applied than other methods for detecting the moisture distribution among numerous specimens and is advantageous for two-dimensional and high-resolution observations. In *C. japonica* heartwood, it is difficult to determine the wetwood boundary in ordinal heartwood with the naked eye, although soft X-ray photography reveals this distinction with ease and precision.

In this study, soft X-ray photography was used to observe water distribution in the stem of *C. japonica* in the green condition for numerous specimens. The features of moisture distribution in the stem of *C. japonica* are described.

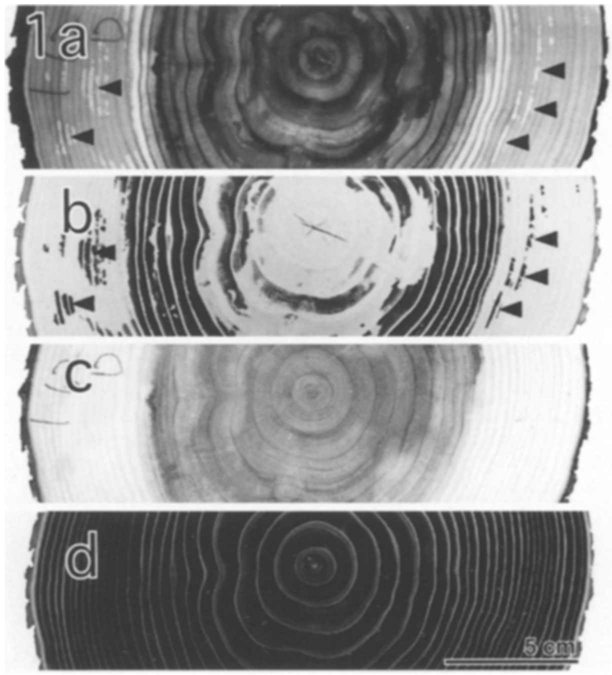

Fig. 1. Optical and X-ray photographs of a single sample section in different moisture states (green or air-dry). **a** Optical, green. **b** X-ray, green. **c** Optical, air-dry. **d** X-ray, air-dry. *Arrowheads* in **a** and **b** show small dehydrated portions in the sapwood. Section no. 62D; section height above ground = 6.0 m; clone name Ashigara-shimo 9. Note: All X-ray photographs in this paper are represented in the negative

Materials and methods

Thirty-five trees were used in this study. Twelve clones, three individual trees per clone (only two trees for one clone) were collected from clonal archives of the *C. japonica* plus-tree at the Forest Tree Breeding Center (Mito, Ibaraki Prefecture, Japan) in February 1995 and January 1996. The age, height, and diameter at breast height of sample trees at harvesting ranged from 30 to 35 years, 12 to 20 m, and 18 to 37 cm, respectively. Several disks (usually seven) of each tree were taken from the stem at various heights. The sampling heights in the stem ranged from 1 to 16 m above the ground.

The sample disks were cut with a band saw into strips 5 cm wide; then cross sections 5 mm thick were prepared from each strip with a disk saw for X-ray photography. Some sample strips were investigated after storage. The strips were covered with aluminum foil, wrapped in a plastic bag, and stored in freezers at −15°C or −30°C.

The prepared sections were placed on X-ray film packs (Kodak x-omat TL ready-pack) directly and irradiated with the soft X-ray irradiator (Softex Co.). Irradiating conditions were 4 min, 15 kVP, 12 mA, and 1.45 m for the distance from the focusing plane to the film. Processed films were observed by the naked eye under transparent light and with an optical microscope to obtain further details.

For this study the terms used have the following meanings: *heartwood*, the portions of xylem with heartwood color (red, reddish brown, or reddish black in *C. japonica*) when specimens were observed with the naked eye (therefore, artificial or false heartwood was included); *wetwood*, the portion in the heartwood where water has accumulated, which shows a higher moisture content than the surrounding regions.

Results and discussion

Water distribution in sapwood, heartwood, and intermediate wood

Figure 1 represents a comparison between the optical views and X-ray photographs of a sample section. Water absorbs soft X-ray light effectively, so a pale area appears on film (as in the figures included here) where there is accumulated water in a portion of the specimen. It is easy to recognize the three xylem layers (i.e., sapwood, heartwood, intermediate wood) by their color in the green sections with visible light. Therefore, the difference in water presence among the three layers was distinguishable by comparing the optical and X-ray photographs. When in-depth discussion of X-ray photography is necessary in this paper, descriptions are based on observations of negatives, as with X-ray film.

Evenly high X-ray absorption was usually observed in sapwood, so it was considered that water distributed almost evenly in sapwood, although marked dehydration was occasionally observed. Part of the dehydrated portions obviously connected to wounds that derived from various sources, e.g., insect attacks or artificial boring (arrowhead, Fig. 2). Other areas of dehydration, the causes of which were impossible to detect, were also found (arrowheads, Fig. 1). In some trees it was observed that most of the free water in the sapwood disappeared because of the suppression or heavy damage from liana or insects. Figure 3, which

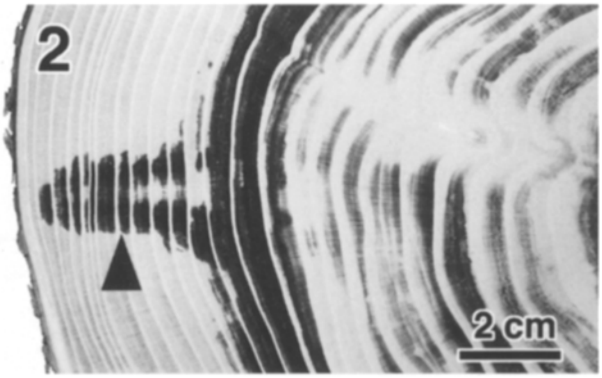

Fig. 2. X-ray photograph of a green section showing dehydration caused by an artificial boring (*arrowhead*) in the sapwood. Section no. 83A; section height above ground = 1.2m; clone name Higashi-shirakawa 5

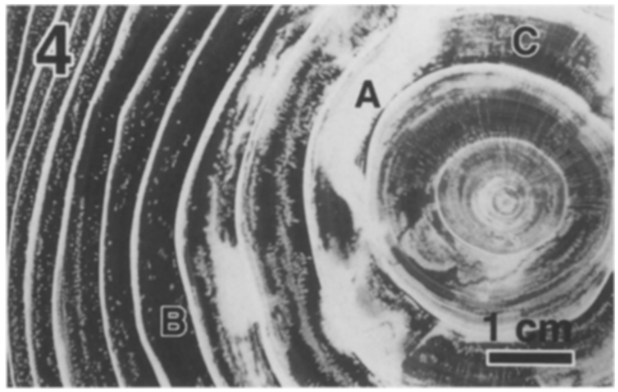

Fig. 4. X-ray photograph of heartwood of a green section, showing maldistribution of water in the heartwood and three types of water presence. *A*, wet area; *B*, dry area; *C*, moderate moisture area. Section no. 12B; section height above ground = 4.0m; clone name Wakamatsu 2

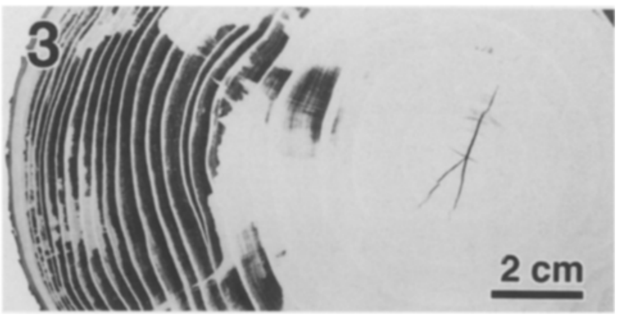

Fig. 3. X-ray photograph of a green section taken from a tree heavily damaged by liana, showing dehydrated sapwood and water-rich heartwood. Section no. 113D; section height above ground = 6.0m; clone name Numata 2

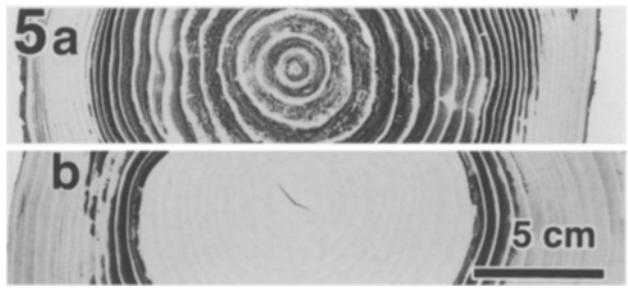

Fig. 5. X-ray photographs of green sections showing even water distribution. **a** Whole heartwood was occupied by the moderate moisture area. Section no. 43A; section height above ground = 1.0m; clone name Yaita 3. **b** Almost of the heartwood was occupied by the wet area. Section no. 103B; section height above ground = 1.9m; clone name Kanra 1

is an X-ray photograph of a tree damaged by liana, demonstrates that most of the sapwood was dehydrated, although a considerable amount of water remained in the heartwood.

The intermediate wood, also called the "white zone" in *C. japonica*, appeared between the sapwood and the heartwood. The intermediate wood was easily recognizable in green sections (Fig. 1a,b) but not in air-dried sections (Fig. 1c,d). X-ray photographs show that the intermediate wood contains a slight amount of water. Water appeared as small scattered specks in the intermediate wood. The sole difference between the sapwood and the intermediate wood in optical and X-ray photographs was the presence of water. The intermediate wood appeared to surround the heartwood in all investigated specimens. Heights in the stem from which the specimens were taken were as low as 1m and as high as 16m. It was suggested that the intermediate wood generally separated water in the heartwood from water in the sapwood; at least intertracheid water flow from the sapwood to the heartwood was not assumed. However, given that there were knots, the intertracheid water connection between the heartwood and the sapwood was estimated in some cases and not in others. Knots make the surrounding xylem too grain-deviated and of high density. Grain deviation and high density made it difficult to distinguish the presence of water on X-ray film. Thus, estimating the presence of an intertracheid water connection between the heartwood and the sapwood in the portion surrounding a knot requires some degree of speculation.

In general, water distributed unevenly and accumulated only partially in heartwood. Figures 4 and 5 represent typical specimens of maldistributed and evenly distributed water, respectively. Portion A in Fig. 4 indicates an area of high absorption on the X-ray. It was believed that water accumulated in portion A in Fig. 4. Three types of water presence in the heartwood were distinguishable (portions of A, B, and C in Fig. 4).

We termed an area of marked X-ray absorption such as that observed in portion A in Fig. 4 a "wet area," an area less absorptive such as portion B in Fig. 4 a "dry area," and an area containing small scattered specks such as portion C in Fig. 4 a "moderate moisture area." The wet area in the heartwood frequently appeared paler than the sapwood on X-ray film. The dry area was rarely present, and when it was distinguishable its size was consistently small. The moderate moisture area appeared in an intermediate state between the wet area and the dry area.

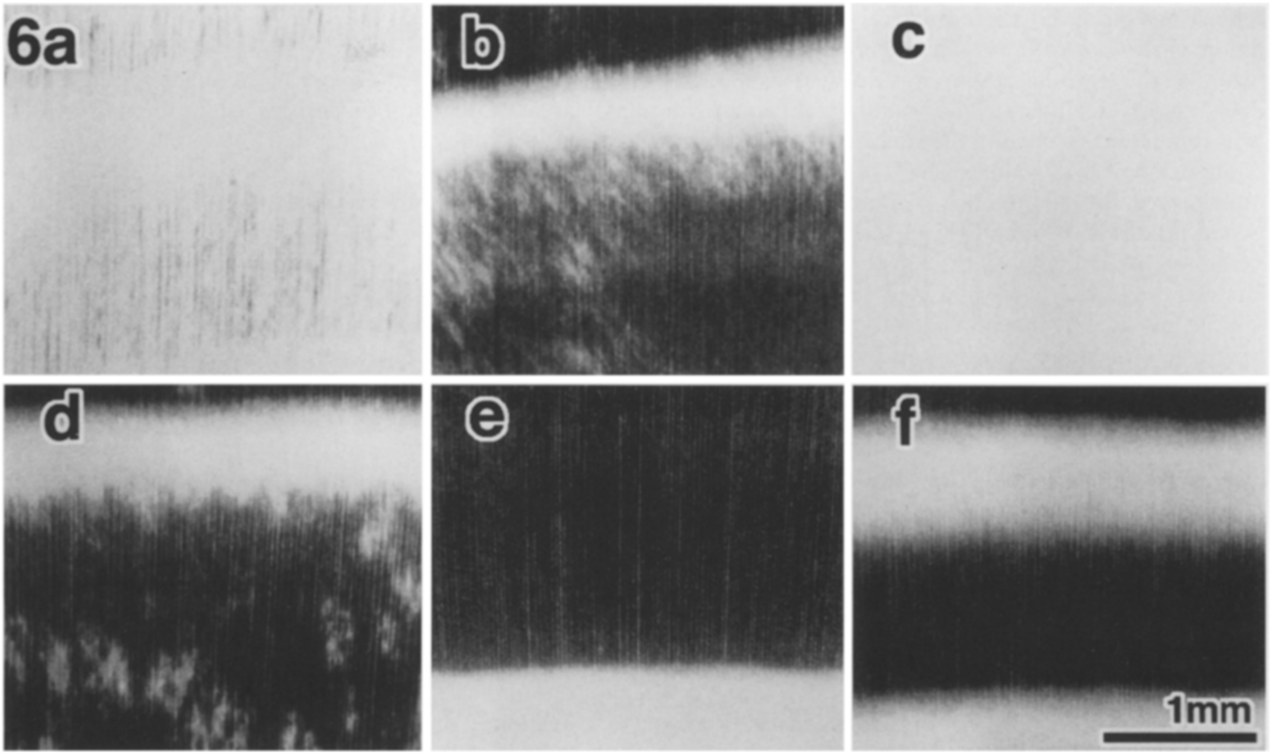

Fig. 6. Micrographs of X-ray films of a sample section. **a–e** Green wood. **a** Sapwood. **b** Intermediate wood. **c–e** Heartwood. **c** Wet area. **d** Moderate moisture area. **e** Dry area. **f** Air-dried section, sapwood. Section no. 12C; section height above ground = 6.0m; clone name Wakamatsu 2

The borders among the three types of heartwood with regard to the presence of water were clear in some cases and not in others. The density and size of small specks in the moderate moisture area were various and changed continuously. Although the borders among the three types of water presence were clear, the borderlines were usually not related to the growth ring boundaries.

In this study, there were no specimens in which the dry area occupied whole heartwood. When a section showed even water distribution in the heartwood, the heartwood was occupied by the moderate moisture area (Fig. 5a) or by the wet area (Fig. 5b).

There was a large variation among trees in terms of the size and form of the wet area in heartwood. The wet area distribution pattern in heartwood generally varied not only among trees but also within a single tree. In a sample tree the heartwood of the lower stem contained a moderate moisture area only, but the higher stem was occupied by a wet area. In contrast, other trees possessed a wetter lower stem and a dryer upper stem. Additional features worth noting in the appearance of the wet area in the heartwood will be described in a later paper.

Microscopic observations on X-ray film

Microscopic observations on X-ray film were made to obtain further details of water distribution. An outline of the tracheid shape on the transverse view in earlywood was easily recognizable in X-ray photographs of air-dried specimens (Fig. 6f) but not in the sapwood (Fig. 6a) or in the wet area in heartwood (Fig. 6c) of green specimens. The recognizable and unrecognizable parts of the tracheid outline were distinguishable in the earlywood of intermediate wood (Fig. 6b) and the moderate moisture area in heartwood (Fig. 6d). In the dry area in heartwood (Fig. 6e), the tracheid outline was as easily recognized as in air-dried specimens. The sole difference between air-dried and green specimens is whether the specimen was dried. In addition, unrecognizable parts of the tracheid outline disappeared after drying in the moderate moisture area and the intermediate wood. It is believed that the difference in X-ray absorption and tracheid outline recognition between air-dried and green specimens are the result of disparate water presence, and it is assumed that the outline of the tracheid corresponds to the tracheid wall. It was thus concluded that the unrecognizable parts of the tracheid outline of the earlywood in green sections contained free water in the tracheid lumina, and the recognizable parts of the tracheid outline contained little free water.

Much higher X-ray absorption occurred consistently in the latewood of green specimens than in air-dried specimens, given that no considerable dehydration caused by wounds was present in the specimen and regardless of the difference among heartwood, sapwood, and intermediate wood. In X-ray photographs of air-dried specimens, it was difficult to recognize the tracheid outline of latewood clearly owing to the limited resolution of X-ray photogra-

phy. The logic used to determine the presence of free water in the earlywood of the green specimen, which made use of whether the tracheid outline was distinguishable, was then hardly applied. However, the boundary of two growth rings was relatively less clear in the wet area than in the intermediate wood, in which the tracheid of earlywood was almost dehydrated. At the boundary between earlywood and latewood in a growth ring of green sections, the difference of depth between them looks, in the photograph, like a boundary of two growth rings, without appearing to change gradually in depth. On the other hand, gradual change of the depth at a transition zone between earlywood and latewood in a growth ring was observed in an air-dried specimen. In addition, latewood of all growth rings appeared in an X-ray photograph to be of the same depth wherever a section included no major dehydration. Therefore, we concluded that free water was usually present in the tracheid lumen of latewood in sapwood, intermediate wood, and heartwood.

In some specimens it was observed that the sapwood was darker – of more depth – on X-ray photographs than the wet area in the heartwood (see Fig. 1 and compare Figs. 6a and 6c). Microscopic observation revealed that this visual characteristic was derived from partial dehydration, seen as columns of black dots in Fig 6a. A pale, thin strip was located among the black dots in the row. It was assumed that the strip corresponded to the tracheid wall, and the black dots were believed to be dehydrated tracheid lumina. The columns of black dots were distributed at random in the sapwood. No such column of black dots was observed in the wet area. It was believed that the presence of this small-scale dehydration in the sapwood and the absence of small-scale dehydration in the wet area caused the darker appearance of the sapwood.

The small specks in intermediate wood and moderate moisture area of heartwood were observed as clusters consisting of dozens of water-saturated tracheids (Fig. 6b,d). The sizes and forms of these clusters varied.

Other authors[15,19,20] investigating the presence of free water in the lumina of xylem elements in some species using cryo-SEM observed that the cell lumina were saturated with ice. There were free-water-saturated cells and water-free cells neighboring each other. Unfortunately, we could not use cryo-SEM or other equipment to make observations at higher resolution. However, based on the careful and considerable X-ray observations in this study, it was concluded that the tracheids located in the sapwood and the wet area, in parts of the intermediate wood and the moderate moisture area, and in terminal parts of growth rings were saturated with water.

Dehydration at sapwood–intermediate wood boundary

Although the sapwood and intermediate wood were easily distinguishable, it was difficult to define a strict border between the two layers. Growth rings, which could not be determined as belonging to the innermost sapwood or outermost intermediate wood, usually contained a narrow dehydrated zone at the inner part of the ring. In the intermediate wood the dehydrated zone within the inner growth ring was wider than that in the neighboring outer ring. In areas of dehydration derived from wounds, it was observed that water was dehydrated first from the initial parts of the rings.

Based on observations in this study, during the formation of heartwood considerable dehydration occurs, first from the initial part of a growth ring and then spreading to the terminal part of the growth ring. Finally, most of the growth ring was dehydrated, though water remained in the outermost terminal part of the growth ring (latewood included). This growth ring was recognized clearly as intermediate wood when observed with the naked eye. This gradual dehydration and water presence in the outermost terminal part of growth rings may make it difficult to define a strict border between sapwood and intermediate wood.

Rewetting at the intermediate wood–heartwood boundary

The intermediate wood, which contains less free water in the lumina of most tracheids in earlywood, was observed in all investigated sections from various heights in the stem. It was believed that all parts of the heartwood had been in a low-moisture state as intermediate wood, and it was concluded that most of the free water in heartwood was present as a result of free water rewetting the tracheid lumen. Ward and Zeikus[5] suggested two origins of wetwood moisture: sapwood transitional and wet-heart types. According to those authors, wetwood of *C. japonica* belongs to the latter type.

It was observed that the pale-colored outermost heartwood was always located outside the wet area. In this study, the statement on which we based our determination of the extent of the heartwood region was that "heartwood is the portion of xylem with heartwood color." Hence we determined that this pale-colored zone was heartwood. The small arrows in Fig. 7 show the outermost heartwood boundary, and the large arrows show the outermost wet area boundary. Regardless of the wet area present in a section, the outermost heartwood was always paler than inner heartwood in the optical view. When there was no wet area in a section, the change of color from the typical intermediate wood to the typical heartwood through this outermost heartwood was gradual, and border definition was difficult. Therefore, it was concluded that heartwood formation was in progress in the pale-colored heartwood, and that rewetting the tracheid lumina of the heartwood occurs after heartwood coloring. In other words, free water rewetted the tracheid lumen of the heartwood near the end of its formation. However, there is a maldistribution of free water, and some sections contained no wet area. Therefore, it was considered that rewetting of the tracheid lumen near the end of the heartwood formation did not always occur. It was believed that the difference in the rewetting process among portions within a tree and among trees was a principal factor in water maldistribution and the appearance of three types of water presence in heartwood.

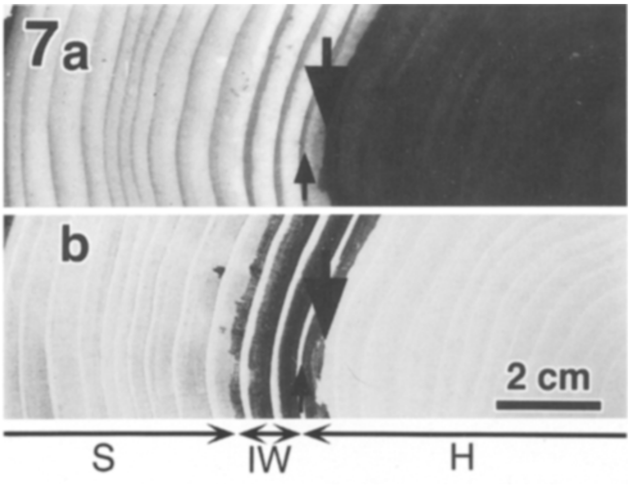

Fig. 7. Photographs of a green section showing pale-colored outermost heartwood. **a** Optical photograph. **b** X-ray photograph. *Large arrow*, outer boundary of wet area; *small arrow*, outer boundary of heartwood; *S*, sapwood; *IW*, intermediate wood; *H*, heartwood. Section no. 103B; see legend for Fig. 5b

Nobuchi et al.[21] also reported that "a pale-colored narrow band surrounds the inner core of the dark-colored heartwood." They believed this zone to be a transitional zone between intermediate wood and heartwood and that the coloring substance in this zone was diffused from the heartwood toward the cambial side. Further investigation and discussion about the relation between the rewetting of wetwood and the heartwood formation of *C. japonica* are needed.

Conclusions

There are three primary conclusions to be drawn from this study.

1. Water in the heartwood of *Cryptomeria japonica* was generally maldistributed, and three types of water presence in the heartwood were distinguished: a wet area, a dry area, and a moderate moisture area.
2. The intermediate wood, which contained little water, always appeared between the sapwood and the heartwood. The appearance of the intermediate wood suggested there was usually no intertracheid water connection between sapwood and heartwood.
3. The presence of free water in the wet area was derived from the rewetting of the tracheid lumina by free water. A difference in the rewetting process among portions in a stem may cause water maldistribution and the differences among the three types of water present in the heartwood.

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